

August 2, 2014

Kyle Lawrence  
CTEH  
5120 Northshore Drive  
N. Little Rock, AR 72118

Re: Lab Project Number: 60174738-002  
Client Project ID: Wet Test

Dear:

Enclosed are the analytical results for sample(s) received by the laboratory. The results relate only to the samples included in this report. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report.

If you have any question concerning this report, please feel free to contact me.

Sincerely,

A handwritten signature in black ink that reads "Tim Harrell". The signature is written in a cursive, flowing style.

Tim Harrell  
[Tim.Harrell@pacelabs.com](mailto:Tim.Harrell@pacelabs.com)  
Technical Director

# Pace Analytical Services, Inc.

808 West McKay, Frontenac, KS 66763

## LABORATORY REPORT:

<b>CLIENT:</b> Kyle Lawrence CTEH 5120 Northshore Drive N. Little Rock, AR 72118 1-314-773-3035	<b>Date Reported:</b> 8-2-14 <b>Date Initiated:</b> 7-31-14 <b>Time Arrived:</b> 10:15 <b>Date Terminated:</b> 8-2-14
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## BIOMONITORING STUDY

### ACUTE TOXICITY

*Permit #*

### FINDING AND CONCLUSIONS:

Acute toxicity testing was performed on duplicate samples of effluent collected from the CTEH (0730WET01) effluent discharge. **Acute toxicity**, as defined by significant mortality for at least one of two aquatic test species during a 48 hour period of exposure, was **detected in Ceriodaphnia exposed to the 100% effluent (AEC), and was not detected in fathead minnows exposed to the 100% effluent. The LC50 for the Ceriodaphnia was >50% and >100% for the Pimephales.** The test species utilized in this test were the water flea, Ceriodaphnia dubia and the fathead minnow, Pimephales promelas. Detailed results of the toxicity testing are provided in the Acute Toxicity Reports. In addition to the acute toxicity testing, water temperature, pH, dissolved oxygen, total hardness, total alkalinity, conductivity, and chlorine determinations were performed on the effluent and control samples.

### SAMPLING PROCEDURES:

CTEH personnel collected a sample at the CTEH (0730WET01) effluent discharge. The sample was preserved with ice and transported to Pace Analytical by commercial carrier.

## INTRODUCTION:

The purpose of this test was to determine the acute toxicity of the CTEH (0730WET01) effluent on the freshwater invertebrate, Ceriodaphnia dubia and the fathead minnow, Pimephalas promelas. These tests were conducted at Pace Analytical Services, Inc., Frontenac, KS.

## TEST ORGANISMS:

Ceriodaphnia dubia - The genetic stock of Ceriodaphnia dubia used in this acute toxicity Test were originally obtained from a private breeder. Ceriodaphnia are cultured in house at Pace Analytical Services, Inc. Culture methods of Ceriodaphnia were obtained from EPA821-C-02-006 November 2002.

Pimephales promelas - The fathead minnows used in this acute toxicity test were cultured in-house at Pace Analytical Services, Inc., Frontenac, KS and/or were obtained from a private breeder. Fathead minnows are maintained at Pace Analytical Services until use for acute toxicity between the ages of 1 and 14 days. Information for culturing fathead minnows was taken from EPA821-C-02-006 November 2002.

## MATERIALS AND METHODS:

Procedures used in the acute toxicity tests are described in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (USEPA, 2002).

CTEH personnel collected the effluent tested from the CTEH (0730WET01) discharge. Testing was performed using a 100% effluent, a series of dilutions, and a synthetic control. **The toxicity test was initiated within 36 hours of sample collection.**

Effluent and synthetic control test solutions were not aerated during the testing period.

## Ceriodaphnia ACUTE METHODS:

This static test was ran using 40 ml glass vials containing 25 ml of test solution. Food was administered before the test. Five Ceriodaphnia neonates (<24 hr old) were randomly selected and placed in each of 4 replicates of test solution. A total of 20 organisms per concentration were tested. Observations of mortality were made at 24 and 48 hours of exposure.

**Pimephales ACUTE METHODS:**

This static toxicity test was conducted using 500 ml polypropylene container as test chambers containing 250 ml of test solution. Food was administered prior to test initiation, but not during the testing period. Ten Pimephales, 1 – 14 days old, from a single spawn, were randomly selected and placed in each of 4 test chambers. A total of 40 organisms were exposed to each test concentration. Observations of mortality were made at 24 and 48 hours of exposure.

**WATER QUALITY METHODS:**

Prior to test initiation, temperature, dissolved oxygen, pH, total alkalinity, total hardness, and total residual chlorine were measured in the effluent and in the controls. At 24 and 48 hours of exposure, temperature, dissolved oxygen, pH, and conductance were measured in the effluent sample and the controls.

**DATA ANALYSIS:**

Statistically significant ( $p < 0.05$ ) mortality is determined by Dunnet's procedure using average percent survival of each test concentration versus the average survival of the controls. If significant mortality occurs, median lethal concentrations (LC50) are calculated using effluent concentrations and their corresponding percent mortality data. The LC50's and the 95% confidence intervals are calculated where appropriate by the Spearman-Kärber method. Statistical analysis is accomplished by following steps in EPA/600/4-90/027E, August 1993 and by use of Toxstat version 3.4.

## RESULTS:

THE Ceriodaphnia MORTALITY RESULTS - There was significant mortality observed of the freshwater invertebrate, Ceriodaphnia dubia, during the 48 hour exposure period to the 100% effluent concentrations. There was no significant mortality in the synthetic control. The LC50 value of the sample to Ceriodaphnia is approximately >50%.

### Ceriodaphnia MORTALITY DATA

#### # ALIVE

CONC.	REP #	0 HOURS	24 HOURS	48 HOURS	% MORT.
SYNTHETIC	1	5	5	5	0
"	2	5	5	5	0
"	3	5	5	5	0
"	4	5	5	5	0
6.25%	1	5	5	5	0
"	2	5	5	5	0
"	3	5	5	5	0
"	4	5	5	5	0
12.5%	1	5	5	5	0
"	2	5	5	5	0
"	3	5	5	5	0
"	4	5	5	5	0
25%	1	5	5	5	0
"	2	5	5	5	0
"	3	5	5	5	0
"	4	5	5	5	0
50%	1	5	5	5	0
"	2	5	5	5	0
"	3	5	5	5	0
"	4	5	5	5	0
100%	1	5	4	3	40
"	2	5	5	3	40
"	3	5	5	4	20
"	4	5	4	3	40

**AVG. MORTALITY @ AEC (100% EFFLUENT) =35%**

**THE Pimephales RESULTS** - Minnows exposed to effluent collected at the CTEH (0730WET01) effluent discharge exhibited no significant mortality in the 100% effluent concentration during the 48 hr exposure period. The synthetic control showed no significant mortality during the testing period. The LC50 value of the effluent to fathead minnows is estimated to be >100%.

CONC.	REP #	0 HOURS	24 HOURS	48 HOURS	% MORTALITY
SYNTHETIC	1	10	10	10	0
"	2	10	10	10	0
"	3	10	10	10	0
"	4	10	10	10	0
6.25%	1	10	10	10	0
"	2	10	10	10	0
"	3	10	10	10	0
"	4	10	10	10	0
12.5%	1	10	10	10	0
"	2	10	10	10	0
"	3	10	10	10	0
"	4	10	10	10	0
25%	1	10	10	10	0
"	2	10	10	10	0
"	3	10	10	10	0
"	4	10	10	10	0
50%	1	10	10	10	0
"	2	10	10	10	0
"	3	10	10	10	0
"	4	10	10	10	0
100%	1	10	10	10	0
"	2	10	9	9	10
"	3	10	10	10	0
"	4	10	10	10	0

*AVG. MORTALITY @ AEC (100% EFFLUENT) = 2.5%*

**WATER CHEMISTRY RESULTS:**

Total residual chlorine (Cl<sub>2</sub>) - The effluent sample from the The CTEH (0730WET01) discharge had <0.1 mg/l detectable level of total residual chlorine upon receipt in the laboratory.

Dissolved Oxygen (D.O.) - Dissolved oxygen reading of the 100% effluent sample was 7.40 mg/l after 30 seconds of vigorous shaking and being raised to the test temperature of 25° C. The D.O. after mixing was less the 1.0. At termination D.O. was 6.50 mg/l in the 100% effluent, which falls into acceptable limits. Aeration was not required in this test.

pH - The pH of the 100% effluent was 7.43 upon receipt in the laboratory and the synthetic control had a 7.72. At termination the pH measurement in the 100% effluent sample was 8.45.

Conductance - The conductance of the effluent sample was 1297 umhos and the synthetic control was 380 umhos.

**INITIAL WATER QUALITY:**

Initial Measurements Synthetic Water

pH	D.O. (mg/l)	Cond. (umhos)	Cl2 (mg/l)	Temp (C)	Hard (mg/l)	Alk (mg/l)
7.72	8.10	380	<0.1	25.0	98	56

Initial Measurements of 100% Effluent

PH	D.O. (mg/l)	Cond. (umhos)	Cl2 (mg/l)	Temp (C)	Hard (mg/l)	Alk (mg/l)
7.43	7.40	1297	<0.1	25.0	238	226

**TEST WATER QUALITY:**

24-hour Water Quality Measurements

EFFLUENT CONC (%)	PH	D.O. (mg/l)	TEMP (C)	COND. (umhos)
Synthetic	7.83	7.80	25.1	377
6.25%	8.01	7.70	25.1	430
12.5%	8.05	7.60	25.1	510
25%	8.15	7.40	25.1	660
50%	8.27	7.20	25.1	905
100%	8.37	6.60	25.1	1442

48-hour Water Quality Measurements

EFFLUENT CONC (%)	PH	D.O. (mg/l)	TEMP (C)	COND. (umhos)
Synthetic	7.93	7.50	25.0	408
6.25%	8.10	7.30	25.0	435
12.5%	8.13	7.20	25.0	539
25%	8.27	7.10	25.0	680
50%	8.32	6.90	25.0	912
100%	8.45	6.50	25.0	1489



**QUALITY ASSURANCE:**

The absence of control mortality during this test indicated the health of the organisms and indicated that any significant mortality in the test concentrations is not due to contaminants or variations in test conditions. Reference toxicity tests are routinely performed by staff members of our Toxicology Department.

**REFERENCE TOXICANT (NaCl)**

**Ceriodaphnia**

**# OF LIVE ORGANISMS**

CONC OF TOXICANT	TEST INITIATION	24 HOUR EXPOSURE	48 HOUR EXPOSURE
3.0 g/l	20	6	0
2.5 g/l	20	15	7
2.0 g/l	20	20	19
1.5 g/l	20	20	20
1.0 g/l	20	20	20

LC50 = 2.38 g/l NaCl

**REFERENCE TOXICANT (NaCl)**

**Pimephales**

**# OF LIVE ORGANISMS**

CONC OF TOXICANT	TEST INITIATION	24 HOUR EXPOSURE	48 HOUR EXPOSURE
10.0 g/l	40	7	0
8.0 g/l	40	35	24
6.0 g/l	40	39	38
4.0 g/l	40	40	40
2.0 g/l	40	40	39

LC50 = 8.22 g/l NaCl

Submitted By: Tim Harrell  
**Timothy Harrell**  
**Technical Director**

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SPECIES TYPE	TEST CHEMICAL	SOLVENT	DATE
Fathead	CTEH 01		8/14

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	40	1	2.5	LESS THAN 0.001
50	40	0	0	LESS THAN 0.001
25	40	0	0	LESS THAN 0.001
12.5	40	0	0	LESS THAN 0.001
6.25	40	0	0	LESS THAN 0.001

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AT A CONFIDENCE LEVEL OF 95 PERCENT, THE BINOMIAL TEST  
SHOWS THAT THE LC50 IS ABOVE 100

THE USEFULNESS OF ANY LC50 CALCULATED FROM THIS SET OF DATA  
IS QUESTIONABLE BECAUSE A CONCENTRATION-EFFECT RELATIONSHIP  
HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (e.g.  
<37 TO>63 OF PERCENT DEAD

NEITHER THE APPROXIMATE LC50 CALCULATION NOR THE MOVING  
AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA.  
EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50  
PERCENT OR THE LOWEST KILLED MORE THAN 50 PERCENT.  
IF THE PROBIT SLOPE IS NEGATIVE, ENTER THE DATA AGAIN  
USING THE NUMBER ALIVE INSTEAD OF THE NUMBER DEAD.

WHEN THERE ARE LESS THAN TWO DIFFERENT CONCENTRATIONS AT  
WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, THE PROBIT  
METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE  
REASONABLE.

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SPECIES  
TYPE

TEST  
CHEMICAL

SOLVENT

DATE

Dubia CTEH 01 8/14

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	7	35	13.15878
50	20	0	0	LESS THAN 0.001
25	20	0	0	LESS THAN 0.001
12.5	20	0	0	LESS THAN 0.001
6.25	20	0	0	LESS THAN 0.001

AT A CONFIDENCE LEVEL OF 95 PERCENT, THE BINOMIAL TEST  
SHOWS THAT THE LC50 IS ABOVE 50

THE USEFULNESS OF ANY LC50 CALCULATED FROM THIS SET OF DATA  
IS QUESTIONABLE BECAUSE A CONCENTRATION-EFFECT RELATIONSHIP  
HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (e.g.  
<37 TO>63 OF PERCENT DEAD

NEITHER THE APPROXIMATE LC50 CALCULATION NOR THE MOVING  
AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA.  
EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50  
PERCENT OR THE LOWEST KILLED MORE THAN 50 PERCENT.  
IF THE PROBIT SLOPE IS NEGATIVE, ENTER THE DATA AGAIN  
USING THE NUMBER ALIVE INSTEAD OF THE NUMBER DEAD.

WHEN THERE ARE LESS THAN TWO DIFFERENT CONCENTRATIONS AT  
WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, THE PROBIT  
METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE  
REASONABLE.

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SPECIES TYPE	TEST CHEMICAL	SOLVENT	DATE
Fathead	CTEH 01		8/14

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	40	1	2.5	LESS THAN 0.001
50	40	0	0	LESS THAN 0.001
25	40	0	0	LESS THAN 0.001
12.5	40	0	0	LESS THAN 0.001
6.25	40	0	0	LESS THAN 0.001

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AT A CONFIDENCE LEVEL OF 95 PERCENT, THE BINOMIAL TEST  
SHOWS THAT THE LC50 IS ABOVE 100

THE USEFULNESS OF ANY LC50 CALCULATED FROM THIS SET OF DATA  
IS QUESTIONABLE BECAUSE A CONCENTRATION-EFFECT RELATIONSHIP  
HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (e.g.  
<37 TO>63 OF PERCENT DEAD

NEITHER THE APPROXIMATE LC50 CALCULATION NOR THE MOVING  
AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA.  
EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50  
PERCENT OR THE LOWEST KILLED MORE THAN 50 PERCENT.  
IF THE PROBIT SLOPE IS NEGATIVE, ENTER THE DATA AGAIN  
USING THE NUMBER ALIVE INSTEAD OF THE NUMBER DEAD.

WHEN THERE ARE LESS THAN TWO DIFFERENT CONCENTRATIONS AT  
WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, THE PROBIT  
METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE  
REASONABLE.

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SPECIES TYPE	TEST CHEMICAL	SOLVENT	DATE
Dubia	CTEH 01		8/14

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
100	20	7	35	13.15878
50	20	0	0	LESS THAN 0.001
25	20	0	0	LESS THAN 0.001
12.5	20	0	0	LESS THAN 0.001
6.25	20	0	0	LESS THAN 0.001

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AT A CONFIDENCE LEVEL OF 95 PERCENT, THE BINOMIAL TEST  
SHOWS THAT THE LC50 IS ABOVE 50

THE USEFULNESS OF ANY LC50 CALCULATED FROM THIS SET OF DATA  
IS QUESTIONABLE BECAUSE A CONCENTRATION-EFFECT RELATIONSHIP  
HAS NOT BEEN DEMONSTRATED OVER A REASONABLE RANGE (e.g.  
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NEITHER THE APPROXIMATE LC50 CALCULATION NOR THE MOVING  
AVERAGE METHOD CAN BE USED WITH THIS SET OF DATA.  
EITHER THE HIGHEST CONCENTRATION KILLED LESS THAN 50  
PERCENT OR THE LOWEST KILLED MORE THAN 50 PERCENT.  
IF THE PROBIT SLOPE IS NEGATIVE, ENTER THE DATA AGAIN  
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WHEN THERE ARE LESS THAN TWO DIFFERENT CONCENTRATIONS AT  
WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, THE PROBIT  
METHOD CANNOT GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE  
REASONABLE.

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